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Structural Studies of Selected Zinc Finger Proteins
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Beamline(s): X4A

Introduction: The Cys_2His_2 zinc finger motif is a modular DNA-binding domain that provides an excellent framework for the design and selection of proteins with novel DNA-binding specificities. We have solved the co-crystal structures of two different 'designer' zinc finger proteins bound to their DNA sites. Zif268-GCN4 is a homodimeric protein constructed by fusing two zinc fingers of Zif268 to the leucine zipper of GCN4 and selecting by phage display the optimal linker between the zinc fingers and the dimerization domain. This protein binds tightly to a palindromic DNA site in which the half sites overlap by two base pairs [1]. P53-ZF is a variant of Zif268 produced by sequential selection [2] that recognizes the P53 binding site. Site selection experiments have shown that its optimal DNA binding site differs from the target site by a single base pair [3]. To study the structural basis of this preference we have solved the structures of P53-ZF bound to DNAs containing both the target and the optimal binding sites.
Methods and Materials: The Zif268-GCN4 binding site was a 13-mer duplex with a single T overhang at each 5' end formed from the self-complementary oligonucleotide 5'-TCCCACGCGTGGG -3'. The complex was formed in 10 mM Bis-Tris Propane (pH 8.0), 1 mM $MgCl_2$. Crystals ($P3_121$, a = b= 87.4Å, c = 118.7Å) were grown by vapor diffusion against 600 mM NH_4OAc. The structure was solved by MIRAS and partially refined at 1.85Å using native and derivative data from a rotating anode source. The final refinement at 1.5Å made use of a native data set collected at X4A. Co-crystals of P53-ZF were grown with three different duplex DNAs, all containing 13 bases per strand. Each of these oligos contained the nine base pair site recognized by the protein flanked on either side by an additional complementary base pair and a T-T mismatch. One complex contained the target site sequence (T at position 9 of the primary strand) and two contained the optimal site (C at position 9). The two 'optimal' oligo sequences differ in the bases flanking the nine base pair core of the protein's binding site. The different co-crystals of P53-ZF are nearly isomorphous ($P3_121$, a = b = 75.5Å, c = 84.5Å) and were grown by vapor diffusion with drops containing equal volumes of complex solution (1 mM complex in 10 mM Bis Tris Propane pH 8, 800 mM NH_4OAc) and well solution (10 mM Bis Tris Propane pH 8.0, 15-20% PEG 6000). The structures were solved by MIRAS and partially refined using data from a rotating anode source but the final high resolution native data sets (1.65Å, 1.85Å and 1.9Å for the target and two optimal structures) were collected at X4A.
Results: The Zif268-GCN4 structure shows the overall architecture of the dimer, the detailed structure of the selected linker and the details of the base-specific protein-DNA interactions. The linker is mostly helical. At its C-terminal end ithe linker acts as an extension to the coiled-coil dimerization domain, making hydrophobic contacts with a symmetry related copy of itself across the dimer interface. In the middle of linker a kink in the helix introduced by Pro 58 changes the direction of the helical axis, allowing the N-terminal end of the linker to fuse seamlessly with the recognition helix of the final Zif268 derived finger. The docking of the zinc finger domains to the DNA is nearly identical to the docking of the corresponding fingers in Zif268, and the details of the interactions of the protein side chains with the DNA bases are similar except in the region where the two DNA half sites overlap. At the two base pair overlap (where no effort was made to optimize the protein sequence) Arg 49 has multiple conformations that involve several different interactions with the two overlapping base pairs. The structures of the P53-ZF complexes show a number of interesting side chain-base interactions never observed before in a crystal structure of a zinc finger-DNA complex, and also give a convincing explanation for the small, but significant, difference in the affinity of the protein for the target and optimal binding sites. The protein has an approximately twofold higher affinity for the optimal site and comparison of the structures shows that in the complex with the target site Glu 124 makes a single direct contact to a DNA base (a hydrogen bond to the C on the complementary strand at position 8 of the 9 base pair core site) but this side chain makes an additional base contact (a second hydrogen bond to the C substituted for the T in the target site on the primary strand at position 7).

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References:
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